Award Number: W81XWH-15-1-0409

TITLE:
4 Birds 1 Stone to Inhibit 5androstane-3alpha,17beta-diol Conversion to DHT

PRINCIPAL INVESTIGATOR:
Michael V. Fiandalo PhD

CONTRACTING ORGANIZATION:
Health Research, Inc.

Buffalo, NY 14263

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4 Birds 1 Stone to Inhibit 5androstane-3alpha,17beta-diol Conversion to DHT

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6. AUTHOR(S)

Michael V. Fiandalo PhD

E-Mail:Michael.Fiandalo@RoswellPark.org

5d. PROJECT NUMBER

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Health Research, Inc.
Buffalo New York 14263

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer growth and survival relies on the interaction between androgen receptor and the testicular androgens, testosterone or dihydrotestosterone (DHT). Men diagnosed with advanced prostate cancer or failure potentially curative therapy are treated with androgen deprivation therapy (ADT). ADT is palliative and CaP recurs. One mechanism that contributes to CaP recurrence is androgen metabolism and our laboratory is studies the primary backdoor androgen metabolism pathway. The terminal step of the primary backdoor androgen metabolism pathway involves the conversion of androstanediol to DHT. There are no inhibitors that inhibit the enzymes, HSD17B6, RDH16, DHRS9 or RDH5, that metabolize androstanediol to DHT. All 4 enzymes have a common conserved catalytic site. The goal of these studies is to identify an inhibitor that impairs all 4 enzymes. During this award period, we have identified the residues responsible for enzyme activity and developed a 3 step inhibitor screen to identify inhibitors against the 4 enzymes.

15. SUBJECT TERMS

Prostate cancer, 3a-oxidoreductase, dihydrotestosterone (DHT), primary backdoor pathway, androstanediol

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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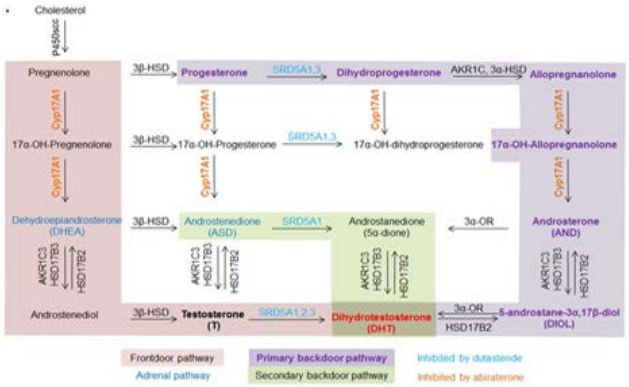
Table of Contents

1. The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

2. **INTRODUCTION:**

Fig. 1 Androgen metabolism pathways to DHT



Prostate cancer (CaP) growth and progression relies on the interaction between androgen receptor (AR) and testicular androgens, testosterone (T) or dihydrotestosterone (DHT). Most men diagnosed with advanced CaP or who failed potentially curative therapy are treated with androgen deprivation therapy (ADT). ADT is palliative and CaP recurs as lethal castration-recurrent/resistant CaP (CRPC). One mechanism that contributes to ADT failure is intratumoral intracrine androgen metabolism, which is defined as the conversion of weak adrenal androgens to T or DHT. There are 3 pathways to DHT synthesis, the frontdoor pathway, the primary and secondary backdoor androgen metabolism pathways (Fig. 1). The terminal step of the primary backdoor pathway involves the conversion of 5androstane3α,17β-diol (androstanediol) to DHT. The conversion of androstanediol to DHT is performed by up to 4 3α-oxidoreductases, HSD17B6, RDH16, DHRS9 and RDH5. Protein sequence alignment using Constraint-based Multiple Protein Alignment Tool showed that all 4 enzymes possess a conserved amino acids involved with catalytic activity. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis showed that combined treatment using catalytic inactive 3α-oxidoreductase mutants and the 5α-reductase inhibitor, dutasteride, decreased DHT levels better than dutasteride alone.

There are no inhibitors against these 4 enzymes used in the clinic to treat CaP. The goal of these studies is to identify an inhibitor against the 3α-oxidoreductases using a three step screen that consists of 1) AR-ARE luciferase, 2) fluorescent-andorgens and 3) mass spectrometry. These preclinical results suggest a new treatment directed against the terminal steps of the frontdoor and primary backdoor pathways may decrease DHT levels better than ADT alone. Further reduction of tissue DHT levels by inhibiting the last steps in intracrine androgen metabolism may improve response to ADT or induce re-remission of castration-recurrent CaP (CRPC) and improve survival of men with advanced CaP.

3. **KEYWORDS:**
    a. 3α-oxidoreductase, androgen deprivation therapy, androgen metabolism, primary backdoor androgen metabolism pathway and dihydrotestosterone

4. **ACCOMPLISHMENTS:**

What were the major goals of the project?

a. Major goal/Specific Aim 1: Determine which catalytic amino acids were critical for enzyme activity (100% completed)
b. Major goal/Specific Aim 2: Identify inhibitors against 3α-oxidoreductases that convert androstanediol to DHT
    i. SOW target date 12 months, 50% completed
c. Major goal/Specific Aim 3: Characterize most promising inhibitor using *in vitro* CaP cell line models and *in vivo* using 3 CaP xenografts

i. SOW target date: 24 months, 0% completed

5. **What was accomplished under these goals?**

A. Year 1 Tasks were copied from the Statement of Work and progress reported for each bullet under each task for each specific aim.

Specific Aim 1: Determine which catalytic amino acids were critical for enzyme activity (100% completed)

Task 1: Summarize and develop manuscript

The significant results of were Specific Aim 1 were:

a. Identified critical amino acid residues for the four 3α-oxidoreductases that convert androstanediol to DHT
b. Showed that inhibition of terminal steps of frontdoor and primary backdoor androgen metabolism lower DHT better than inhibition of either pathway alone
    i. Manuscript under review by authors for submission
    ii. Manuscript should be submitted for journal review by end September 2016

Specific Aim 2: Identify inhibitors against 3α-oxidoreductases that convert androstanediol to DHT

Task 1: Identify inhibitors against 3α-oxidoreductases that convert androstanediol to DHT

Three screen approach to identify inhibitors: 1) AR-ARE luciferase to assess inhibitors that reduce AR activity; 2) Fluorescence-based assay that assess androgen metabolism inhibition; and 3) Liquid chromatography-tandem LC-MS/MS to confirm androgen metabolism inhibition

Subtask 1: Develop fluorescent-androgens and validate metabolism by 3α-oxidoreductases using LC-MS/MS

Fluorescent-androgens (DHT-coumarin, [DHT-C] androstanediol-coumarin [DIOL-C], androsterone-C [AND-C] and androstanedione-C [5α-dione-C]) were generated by collaborator Dr. David Watt (University of Kentucky, Lexington, KY) and characterized in our laboratory. CaP cells were treated with fluorescent-androgens and CaP cell growth rate and AR activation were assessed using MTT or qRT-PCR, respectively. Data generated from these experiments showed VCaP cell growth persisted after androgen deprivation using serum-free complete media (SFM) after treatment with DHT-C (Fig. 2A). DHT-coumarin stimulated AR activation in VCaP and LAPC-4 cell lines (Fig. 2B).

PC-3 cells that contained empty plasmid (control) or expressed either wild-type or catalytic impaired double mutant (DM) RHD16 were treated with AND-C or 5α-dione-C. Fluorescent-androgen metabolism was assessed using LC-MS/MS. LC-MS/MS data showed that wild-type RDH16 (RDH16 WT) metabolized AND-C to 5α-dione-C (Fig. 2C). LC-MS/MS data that showed conversion of DIOL-C to DHT-C will be included in the manuscript. The manuscript is in preparation for submission by the end of 2016.

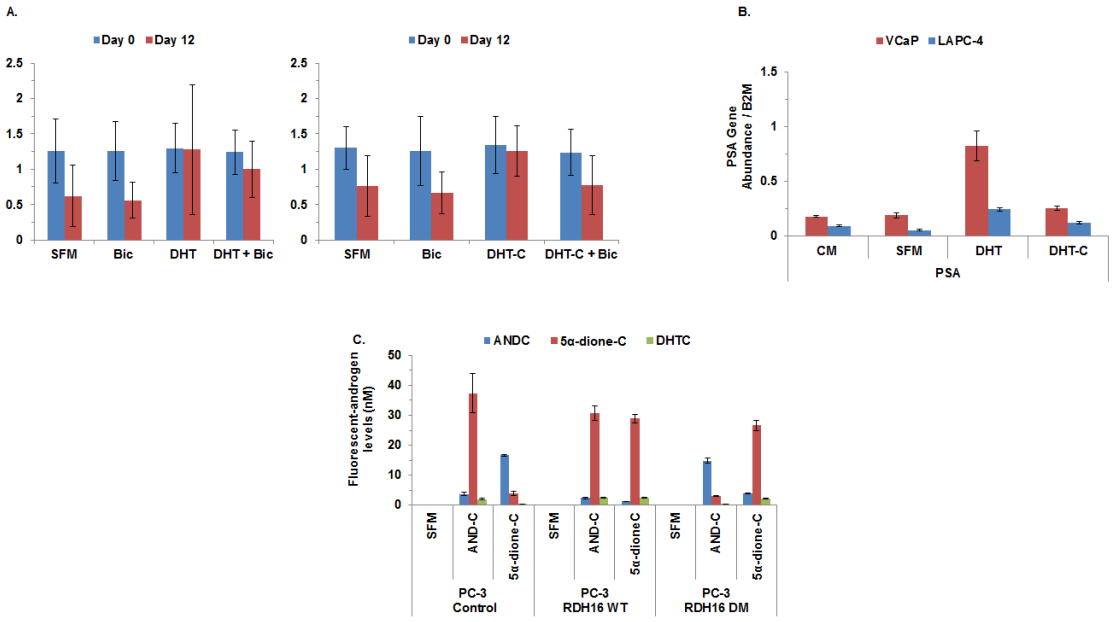


Fig. 2 Characterization of fluorescent androgens: VCaP cells were treated for 12 days using SFM alone or SFM that contained 1µM bicalutamide, 10nM DHT or DHT + 1 µM BIC (left panel). VCaP cells were treated with SFM, 1 µM Bic, 10 nM DHT-coumarin (DHT-C) serum-free complete media (SFM) alone or SFM that contained 1 uM bicalutamide (Bic), 10 nM DHT or 10 or 20 nM DHT-C + Bic (right panel). qRT-PCR was performed using VCaP or LAPC-4 cells treated with complete media that contains 10% FBS(CM), SFM, 10 nM DHT or 10 nM DHT-C. Treatment with DHT or DHT-C stimulated PSA gene transcription (B). LC-MS/MS data showed 5α-dione was produced only when PC-3 cells expressed wild-type RDH16 (RDH16 WT). Experiments were performed in triplicate.

Subtask 2 Screen inhibitors using AR-ARE luciferase and fluorophore-based assay

A total of 12 PC-3 cell lines were established that expressed combinations of wild-type or double mutant RDH16, AR-green fluorescent protein (GFP) or ARE-luciferase were established (Table 1). PC-3 cells that expressed wild-type RDH16, AR-GFP and ARE-luciferase were tested by our laboratory and the Roswell Park Cancer Institute Small Molecule Screening Facility (SMSF). PC-3 cells that express wild-type RDH16, AR-GFP and ARE-luciferase produced signal when cells were treated with 100 nM androstanediol (DIOL) or 100 nM DHT (Fig. 3A).

Optimization of the AR-ARE lucifierase assay by our laboratory and SMSF will be completed by the end of September 2016. The AR-ARE luciferase screen will be completed by end October 2016 and 2 manuscripts will be generated by end December 2016. Inhibitors identified using the AR-ARE luciferase assay will be tested using the fluorescent-based assay and LC-MS/MS will be performed on inhibitors that passed both the luciferase and fluorescent-based assays.

Table 1: PC-3 AR-ARE luciferase cell lines		
Cell lines		Purpose
1	PC-3	Transfection control
2	PC-3 AR	Control that produces no signal when AR is stimulated with DIOL or DHT
3	PC-3 ARE	Control that produces no signal in absence of AR and cells are treated with DIOL or DHT
4	PC-3 AR, ARE	Control that produces signal when cells are treated with DHT, not DIOL
5	PC-3 RDH16	Control that produces no signal when cells are treated with DIOL or DHT
6	PC-3 RDH16, AR	Control that produces no signal when cells are treated with DIOL or DHT
7	PC-3 RDH16, ARE	Control that produces no signal when cells are treated with DIOL or DHT

8	PC-3 RDH16, AR and ARE	PC-3 cells that produce signal when cells are treated with DIOL or DHT
9	PC-3 RDH16 DM	Match to RDH16 WT, same function as 5
10	PC-3 RDH16 DM, AR	Match to RDH16 WT, same function as 6
11	PC-3 RDH16 DM, ARE	Match to RDH16 WT, same function as 7
12	PC-3 RDH16 DM, AR and ARE	PC-3 cells that produce signal when cells are treated with DHT and not DIOL

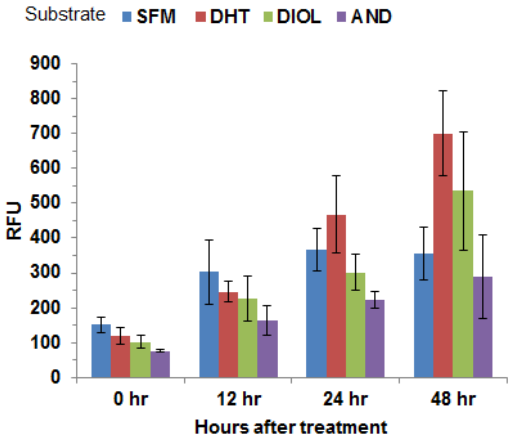


Fig. 3 Luciferase signal (relative light units [RFU]) was produced when PC-3 cell cells that stably expressed RDH16 WT, AR-GFP or ARE were treated with 100 nM DIOL or 100 nM DHT. Experiments were performed in triplicate.

Specific aim 3 work will begin once an inhibitor is identified

6. **What opportunities for training and professional development has the project provided?**

   a. Training opportunities:
      ii. Flow Cytometry Core Facility:
         1. ImageStream and fluorescent microscopy equipment usage and data analysis software training courses
      iii. Small Molecules Core Facility: Drug screen design, development and execution
      iv. Training Dr. James L. Mohler (Mentor), weekly meetings for:
         1. Data analysis and writing manuscripts
         2. Career award development
         3. Grant writing
      v. Training with Dr. Adam Kisailus, Associate Dean of Educational Affairs
         1. Taught graduate level courses
         2. Post-doctoral grant writing program idea development, implementation and execution
         3. Annual genitourniary seminar presentation, monthly journal club presentations, weekly laboratory meetings and presented at international and national conferences

7. **How were the results disseminated to communities of interest?**

   a. Nothing to report

8. **What do you plan to do during the next reporting period to accomplish the goals?**

a. The AR-AR-luciferase screen will be completed by the end of October and the fluorescent-based assay will begin. LC-MS/MS will be performed on samples by the end of the 2016 and *in vivo* studies will begin 2017.

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9. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

a. Nothing to report

10. **What was the impact on other disciplines?**

a. Nothing to report

11. **What was the impact on technology transfer?**

a. Nothing to report

12. **What was the impact on society beyond science and technology?**

a. Nothing to report

13. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

a. The original experiment designed planned to use CV-1 monkey kidney cell line to develop the luciferase and fluorescent-based assays. However, experiments using the cell line produced data that showed CV-1 cells interfere with conversion of androstanediol to DHT. Therefore, the PC-3 cell line, which does not perform endogenous androgen metabolism or express androgen receptor, was used for the luciferase and fluorescent-based assays.

b. The original strategy was to transiently express 3α-oxidoreductases of interest, androgen receptor and ARE. Instead stable cell lines were generated and used for luciferase and fluorescent-based assays.

14. **Actual or anticipated problems or delays and actions or plans to resolve them**

a. Progress was delayed due to generation of expression plasmids that contained different selection markers to generate stable cell lines. Generation of AR or AR-GFP expression plasmids proved challenging because AR gene sequence is large and was prone to nucleotide mutation and it look longer than expected to generate wild-type AR plasmids.

b. Progress was delayed because initially CV-1 cells that stably expressed wild-type RDH16, AR-GFP and ARE were established and data generated using those cells showed this was not an appropriate model to perform luciferase or fluorescent-based assays. Therefore, PC-3 cells that stably express plasmids of interest were generated**.**

15. **Changes that had a significant impact on expenditures**

a. Nothing to report

16. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

    a. **Significant changes in use or care of human subjects:** Nothing to report
    b. **Significant changes in use or care of vertebrate animals:** Nothing to report
    c. **Significant changes in use of biohazards and/or select agents:** Nothing to report

17. **PRODUCTS:**

    a. Nothing to report

18. **Publications, conference papers, and presentations**

    a. Manuscripts are in preparation

Journal publications.

    b. Same response as 18

19. **Books or other non-periodical, one-time publications.**

    a. Nothing to report

20. **Other publications, conference papers, and presentations.**

    a. American Urologic Association (AUA) "Four birds one stone to inhibit 5androstane-3α,17β-diol conversion to DHT" **Michael V. Fiandalo**, James L Mohler; Poster Presentation May 5-12, 2016 San Diego, CA, USA. Won: Best poster
    b. Society for Basic Urologic Research (SBUR) "Four birds one stone to inhibit 5androstane-3α,17β-diol conversion to DHT" **Michael V. Fiandalo**, James L Mohler; Poster Presentation; November 12-15, 2015 Ft. Lauderdale, FL, USA
    c. Northeastern Section Annual Meeting "Four birds one stone to inhibit 5androstane-3α,17β-diol conversion to DHT" **Michael V. Fiandalo**, James L Mohler; Poster Presentation October 29-31, 2015 Quebec City, Canada
    d. ESURSBUR 11th World Congress on Urological Research "Four birds one stone to inhibit 5androstane-3α,17β-diol conversion to DHT" **Michael V. Fiandalo**, James L Mohler; Poster Presentation September 10-12, 2015 Nijmegen, The Netherlands. Won: Travel award

21. **Website(s) or other Internet site(s)**

    a. Nothing to report

22. **Technologies or techniques**

    a. Nothing to report

23. Inventions, patent applications, and/or licenses

Patent Application Type: Provisional
Application Number: 62/313,261
Application Filing Date: March 25, 2016
Title: Inhibition of Catalytic Site Common to Multiple 3 Alpha-Oxidoreductases for Treatment of Prostate Cancer

24. **Other Products**

a. Nothing to report

25. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	James L. Mohler MD	Michael V. Fiandalo PhD
Project Role:	Mentor	Mentee
Researcher Identifier (e.g. ORCID ID):	0000-0002-7726-3795	0000-0002-3558-2712
Nearest person month worked:	12	12
Contribution to Project:	Project Mentor	Mentee
Funding Support:	Will be updated with point 30 below	DoD Award W81XWH-15-1-0409

26. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

James L. Mohler
Previously active, now completed:
Title: Prostate Cancer: Transition to Androgen Independence, Core A: Administration (Mohler – PI) No Cost Extension
Time Commitments: 1.16 calendar months
Supporting Agency: National Cancer Institute P01-CA77739
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Mark Kramer, Administrative Director, UNC Lineberger Comprehensive Cancer Center Campus Box 7295
102 Mason Farm Road, Chapel Hill, NC 27599-7295, Phone: (919) 966-0233, Fax: (919) 966-3015, mkramer@med.unc.edu
Performance Period: 04/01/2005-03/31/2015
Level of Funding: $519,640
Brief description of project's goals: Renewal of an administrative core that provides the leadership for the overall Program Project in the daily execution of administrative matters common to the three projects and ImmunoAnalysis and Tumor Management Core B.
List of specific aims:

The objective of the Administration Core A is to provide leadership, direction and administrative services for the purposes of enhancing research productivity and maintaining a stimulating research environment conducive to study of prostate cancer biology. Administration Core A will foster exchange of ideas and promote collaboration through its interactions with the Project Leaders and research groups. A major effort will be to encourage and facilitate collaboration in translational research among investigators within the Program Project and other investigators within or outside UNC-Lineberger Comprehensive Cancer and Roswell Park Cancer Institute. Administration Core A will have direct responsibility for organization and facilitation of the monthly research conferences and annual review of the Program Project by the 5 external consultants. Administration Core A will monitor activities of ImmunoAnalysis and Research Specimen Management Core B, in particular, and the entire program, in general, to improve the efficiency and effectiveness of the entire program.
Overlap: None

Title: Prostate Cancer: Transition to Androgen Independence, Core B: ImmunoAnalysis and Tumor Management (French – PI)
Time Commitments: 0.48 calendar months
Supporting Agency: National Cancer Institute P01-CA77739
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Mark Kramer, Administrative Director, UNC Lineberger Comprehensive Cancer Center Campus Box 7295
102 Mason Farm Road, Chapel Hill, NC 27599-7295, Phone: (919) 966-0233, Fax: (919) 966-3015, mkramer@med.unc.edu
Performance Period: 04/01/2005-03/31/2015
Level of Funding: $903,203
Brief description of project's goals: Renewal of a core that serves two primary functions to the three projects: Core B is involved in all aspects of clinical specimen and prostate cancer xenograft management and Core B processes and stores the invaluable prostate biopsy specimens obtained from men with advanced prostate cancer prior to and at regular intervals after beginning androgen deprivation therapy.
List of specific aims:
The ImmunoAnalysis and Research Specimen Management Core B will provide 3 primary services to the Program Project.
1. Core B will provide high quality, reliable and cost-effective technical services to participants of the Program Project for immunohistochemistry and quantitative image analysis.
2. Core B will manage the research specimens critical to the conduct of the research proposed by the Program Project.
3. Core B will provide expertise in biostatistics and genitourinary pathology.

Overlap: None

Title: Genetic variations in mitochondria and prostate cancer aggressiveness and progression in Caucasian and African American men (PI – Zhao)
Time Commitments: 0.60 calendar months
Supporting Agency: Department of Defense
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Sherie Wesley, Research Contract Specialist, Legal Services Department, LEGAL SERVICES, Unit 537, P. O. Box 301439, Houston, Texas 77230-1439
Email: swesley@mdanderson.org **Phone**: 713.794.1507 **Fax**: 713.792.6878
Performance Period: 07/01/2012-06/30/2015
Level of Funding: $936,256
Brief description of project's goals: The hypothesis is that genetic variations (sequence and copy number) in mtDNA are associated with prostate cancer aggressiveness at diagnosis and prostate cancer progression**.** The proposed study will represent the first study to address the roles of mtDNA variations in prostate cancer aggressiveness and progression as well as racial difference.

List of specific aims:
1. Evaluate whether genetic variations in mtDNA are associated with aggressive tumor characteristics of prostate cancer at diagnosis and progression of prostate cancer in CA and AA men, and whether the associations are different between CA and AA men.
2. Evaluate whether mtNDA CNVs are associated with aggressive tumor characteristics of prostate cancer at diagnosis and progression of prostate cancer in CA and AA men, and whether the associations are different between CA and AA men.
3. Exploratory Aim: Perform whole mitochondrial DNA sequencing to identify novel genetic variants in AA and CA prostate cancer patients.

Overlap: None

Previously pending, now active:
Title: Cholesterol Lowering Intervention for Prostate Cancer Active Surveillance/Jr. Faculty Award to Alliance NCORP Research Base – Pilot Project (Kim/Mohler - PIs)
Time Commitments: 0.60 calendar months
Supporting Agency: Cedars/NCI
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Subcontract with Cedars Sinai. Cedars-Sinai Medical Center, Attention: Margaret Jenkins, Administrative Program Coordinator Department of Surgery, Research Division, 8635 W. 3rd Street, Suite 973W, Los Angeles, CA 90048 margaret.jenkins@cshs.org
Performance Period: 04/01/2015 – 07/31/2016
Level of funding: $93,955 (sub contract)
Brief description of project's goals: The proposed research tests the hypothesis that intensive cholesterol lowering will decrease the growth rate of benign and malignant prostate epithelium. The proposed research could provide the data necessary to justify a phase III clinical trial to address one of the major problems in urologic oncology how to prevent the progression of low risk prostate cancer to provide men higher levels of confidence for selection of active surveillance.
Overlap: None

Title: A Small-Molecule Inhibitor of the Terminal Steps for Intracrine Androgen Synthesis in Advanced Prostate Cancer (Mohler)
Time Commitments: .975 calendar months
Supporting Agency: NCI-1R21CA205108-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Nicole Franklin, Grants Management Specialist, National Cancer Institute, 9609 Medical Center Drive, West Tower, Room 2W556, Bethesda, MD 20892 (regular mail), Phone: 240-276-5210, Email: nicole.franklin@nih.gov
Performance Period: 04/10/2016-04/09/2018
Level of Funding: $ 416,398
Brief description of project's goals: This research seeks to explore if a small-molecule inhibitor of the catalytic site shared by the five 3α−oxidoreductases will decrease T and DHT metabolism through the frontdoor and backdoor pathways.
List of specific aims:
1. Identify a candidate inhibitor against the catalytic site shared by the five 3α−oxidoreductases
2. Synthesize and test re-designed candidate inhibitors and conduct PK/PD and toxicity studies to produce a lead compound inhibitor of the five 3α−oxidoreductases
3. Determine whether the inhibitor of the 3α−oxidoreductases decreases tissue T and DHT levels and impairs CRPC growth

Overlap: None

End date extended:

Title: Prostate Cancer: Transition to Androgen Independence, Project 1: Interference with the Androgen Receptor and Its Ligands in Recurrent Prostate Cancer (French - PI)
Time Commitments: 0.60 calendar months
Supporting Agency: National Cancer Institute P01-CA77739
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Mark Kramer, Administrative Director, UNC Lineberger Comprehensive Cancer Center Campus Box 7295 102 Mason Farm Road, Chapel Hill, NC 27599-7295, Phone: (919) 966-0233, Fax: (919) 966-3015, mkramer@med.unc.edu
Performance Period: 04/01/2005-03/31/2017 (NCE)
Level of Funding: $2,292,618
Brief description of project's goals: Renewal of a project that tests the hypothesis that recurrence of prostate cancer during androgen deprivation therapy can be prevented or delayed by preventing the accumulation of tissue androgens and/or inhibiting the androgen receptor.
List of specific aims:
1. Prevent the changes in androgen metabolism that provide AR ligand(s) in the immediate post-castration period
2. Degrade AR ligand(s) formed in the immediate post-castration period
3. Diminish or eliminate AR in the immediate post-castration period

Overlap: None

Title: Diet changes among prostate cancer patients under expectant management (Marshall - PI)
Time Commitments: 0.60 calendar months
Supporting Agency: National Cancer Institute
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Program Official: Howard L. Parnes, Email: hp24c@nih.gov Phone: 301-594-0920 Fax: 301-435-1564
Performance Period: 09/28/2009-01/31/2017 (NCE)
Level of Funding: $55,818
Brief description of project's goals: The focus of this study is to assess whether a diet emphasizing plant consumption decreases the probability that low grade, low-volume prostate cancer (LGLV)in expectant management(EM)patients progresses to a more aggressive form of cancer that merits active treatment. The intervention will be conducted through one of the leading cooperative oncology research groups: Cancer and Leukemia Group B (CALGB).
List of specific aims:
1. Assess the effect of a telephone-based dietary intervention on PSA, PSA doubling time, Gleason score and tumor extension in LGLV prostate cancer patients treated with EM.
2. Assess the effect of a telephone-based dietary intervention on treatment seeking, anxiety and coronary heart disease in prostate cancer patients treated with EM.

Overlap: None

Title: Defining intra- and intertumoral genomic heterogeneity in prostate cancer (Mohler - PI)
Time Commitments: 0.60 calendar months
Supporting Agency: Roswell Park Alliance Foundation
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Judith Epstein, Director Grants & Foundation Office, Elm & Carlton Streets, Research Studies Center Room 234, Buffalo, NY 14203, Judith.Epstein@RoswellPark.org
Performance Period: 12/10/2013-12/31/2016
Level of funding: $92,384
Brief description of project's goals:
Intra- and inter-tumoral CaP genomic heterogeneity necessitates extensive sampling of a radical prostatectomy specimen.

List of specific aims:
1. Determine intra- and inter-tumoral heterogeneity in CaP's mutational landscape using whole exome sequencing to determine heterogeneity within and among CaP foci derived from radical prostatectomy specimens from patients with high-risk disease who are expected to develop metastatic disease and require ADT
2. Define intra- and inter-tumoral CaP heterogeneity in structural gene rearrangement and gene expression patterns using RNA-Seq and RNA derived from the same CaP samples used in Aim 1

Overlap: None

Title: Deprive prostate cancer of DHEAS to prevent castration-recurrent prostate cancer (Wu – PI)
Time Commitments: 0.12 calendar months
Supporting Agency: NIH/NCI 1R21CA191895-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Viviana Knowles, 9609 Medical Center Drive, West Tower, Bethesda, MD 20892, phone: 240-276-5157, viviana.knowles@nih.gov
Performance Period: 09/17/2014-08/31/2017 (NCE)
Level of Funding: $419,884
Brief description of project's goals: This research seeks to address the racial differences in prostate cancer aggressiveness from a biological perspective.
List of specific aims:
1. Characterize the expression of STS and potential STS regulators in CRPC
2. Evaluate the value of targeting DHEAS usage by prostate cancer cells to prevent post-castration tumor growth
3. Identify DHEAS uptake mechanisms

Overlap: None

Michael V. Fiandalo
Previously active, now completed:
Title: Prostate Cancer: Transition to Androgen Independence, Project 1: Interference with the Androgen Receptor and Its Ligands in Recurrent Prostate Cancer (French - PI)
Time Commitments: 12.00 calendar months
Supporting Agency: National Cancer Institute P01-CA77739
Name and address of the Funding Agency's Procuring Contracting/Grants Officer:
Mark Kramer, Administrative Director, UNC Lineberger Comprehensive Cancer Center Campus Box 7295 102 Mason Farm Road, Chapel Hill, NC 27599-7295, Phone: (919) 966-0233, Fax: (919) 966-3015, mkramer@med.unc.edu
Performance Period: 04/01/2005-03/31/2015
Level of Funding: $2,292,618
Brief description of project's goals: Renewal of a project that tests the hypothesis that recurrence of prostate cancer during androgen deprivation therapy can be prevented or delayed by preventing the accumulation of tissue androgens and/or inhibiting the androgen receptor.
List of specific aims:
4. Prevent the changes in androgen metabolism that provide AR ligand(s) in the immediate post-castration period
5. Degrade AR ligand(s) formed in the immediate post-castration period
6. Diminish or eliminate AR in the immediate post-castration period

Overlap: None

Previously pending, now active:

Title: A Small-Molecule Inhibitor of the Terminal Steps for Intracrine Androgen Synthesis in Advanced Prostate Cancer (Mohler)
Time Commitments: .975 calendar months
Supporting Agency: NCI-1R21CA205108-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Nicole Franklin, Grants Management Specialist, National Cancer Institute, 9609 Medical Center Drive, West Tower, Room 2W556, Bethesda, MD 20892 (regular mail), Phone: 240-276-5210, Email: nicole.franklin@nih.gov
Performance Period: 04/10/2016-04/09/2018
Level of Funding: $ 660,315
Brief description of project's goals: This research seeks to explore if a small-molecule inhibitor of the catalytic site shared by the five 3α−oxidoreductases will decrease T and DHT metabolism through the frontdoor and backdoor pathways.
List of specific aims:
Aim 1. Identify a candidate inhibitor against the catalytic site shared by the five 3α−oxidoreductases
Aim 2. Synthesize and test re-designed candidate inhibitors and conduct PK/PD and toxicity studies to produce a lead compound inhibitor of the five 3α−oxidoreductases
Aim 3. Determine whether the inhibitor of the 3α−oxidoreductases decreases tissue T and DHT levels and impairs CRPC growth
Overlap: There is no overlap because the trainee funded effort on the R21 begins after the training grant funded effort ends.

David Watt
Previously pending, now active:
Title: Suppression of Prostate Tumor Growth and Metastasis by Inhibition of Vimentin (Rangnekar, Watt and Zhan Co-PIs)
Time Commitments: .55 calendar months
Supporting Agency: National Cancer Institute, Bethesda, MD (NIH R01 CA187273)
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Romy Reis, National Cancer Institute, National Institutes of Health, Rockville, MD, telephone: 240-276-6316; email: mondesir@mail.nih.gov.
Performance Period: 07/01/2015-06/30/2020
Level of Funding: $
Brief description of project's goals: The objective of this grant is to develop new 3-arylquinolones and related compounds for the inhibition of prostate tumor growth and metastasis by inhibiting vimentin and promoting the release of the tumor suppressor, Par-4. Watt's specific role (Note: according to the notice of award this is not a multiple PI grant): Oversee the design and synthesis of "small molecules" that promote Par-4 secretion.
List of specific aims:
Aim 1. (A) Develop structure-activity relationships within arylquins to identify potent analogs that induce Par-4 secretion in diverse normal cells in cell culture and mice as well as paracrine apoptosis of cancer cells in cell culture; and (B) Study Arylquin-1 and analogs to identify compounds that best induce paracrine apoptosis of primary tumors and xenografts in vivo via Par-4 secreted by the normal cells.
Aim 2. (A) Identify the interaction domain(s) of Par-4 and vimentin; and (B) Determine the mechanism by which Arylquin-1 and its analogs inhibit vimentin binding to Par-4 and promote the secretion of Par-4
Aim 3. (A) Determine whether arylquins inhibit motility, invasion and EMT via vimentin-dependent mechanisms in cell culture models; and (B) Determine whether arylquins inhibit prostate cancer metastasis to the lung and other tissues and the growth of prostate cancer cells at the bone in mouse models.
Overlap: None

Title: A Small-Molecule Inhibitor of the Terminal Steps for Intracrine Androgen Synthesis in Advanced Prostate Cancer (Mohler)
Time Commitments: .48 calendar months
Supporting Agency: NCI-1R21CA205108-01
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: Nicole Franklin, Grants Management Specialist, National Cancer Institute, 9609 Medical Center Drive, West Tower, Room 2W556, Bethesda, MD 20892 (regular mail), Phone: 240-276-5210, Email: nicole.franklin@nih.gov
Performance Period: 04/10/2016-04/09/2018
Level of Funding: $ 660,315
Brief description of project's goals: This research seeks to explore if a small-molecule inhibitor of the catalytic site shared by the five 3α−oxidoreductases will decrease T and DHT metabolism through the frontdoor and backdoor pathways.
List of specific aims:
Aim 1. Identify a candidate inhibitor against the catalytic site shared by the five 3α−oxidoreductases
Aim 2. Synthesize and test re-designed candidate inhibitors and conduct PK/PD and toxicity studies to produce a lead compound inhibitor of the five 3α−oxidoreductases
Aim 3. Determine whether the inhibitor of the 3α−oxidoreductases decreases tissue T and DHT levels and impairs CRPC growth
Overlap: None

Title: Lafora Epilepsy - Basic mechanisms to therapy (Gentry)
Time Commitments: .24 calendar months
Supporting Agency: National Institute of Neurological Disorders and Stroke (R01 CA187273)
Name and address of the Funding Agency's Procuring Contracting/Grants Officer: William Benzing, National Institutes of Health, Neuroscience Center Room 3204, 6001 Executive Blvd., MSC 9529, Bethesda, MD 20892-9529; Email: benzingw@mail.nih.gov; Telephone: 301-496-9223
Performance Period: 07/01/2016-06/31/2021
Level of Funding: $
Brief description of project's goals: The objectives are to study Lafora disease (LD), which is a fatal, progressive myoclonic epilepsy and to translate our findings into treatments and cures. Watt's specific role: co-Director of Medicinal Chemistry Core with participation in Projects 1, 2 and 3.
List of specific aims:
Project 1. Personalized diagnosis - defining how glycogen metabolism and proteostasis impact LD:
Aim 1: Utilize a structure/function analysis of laforin and malin to diagnose patient-specific mechanisms.
Aim 2: Targeting proteostasis to treat Lafora disease.
Aim 3: Establish patient-specific treatments of LD Premature Termination Codons (PTCs).
Project 2. Genome editing, mRNA suppression and glycogen chain termination to inhibit glycogen storage as therapy for Lafora disease (LD).
Aim 1: Treat LD mice with AAV9-mediated CRISPR/Cas9 knockout of the GS and PTG genes.
Aim 2: Treat LD mice with with antisense oligonucleotides (ASOs) against GYS1 and PTG. ASOs employ Watson-Crick base pairing between an RNA target receptor and a complementary oligonucleotide to inhibit target function.
Aim 3: Determine whether glucose derivatives can chain-terminate glycogen synthesis to prevent LD. Glycogen chains elongate through the formation of α1-4 bonds between glucose units.
Project 3: Suppressing glycogen storage with small molecule inhibitors as a therapeutic approach to Lafora disease.
Aim 1. Identification and validation of small molecule glycogen synthase inhibitors.
Aim 2. Medicinal chemistry optimization.
Aim 3. Cell-based assay of glycogen accumulation, toxicity and preliminary pharmacokinetics.
Aim 4. Testing inhibitors of glycogen accumulation in mouse models of Lafora disease

Project 4. Defining the therapeutic window for the treatment of Lafora disease (LD).
Aim 1: Determine the effect of the abolition of glycogen synthesis once LD has developed.
Aim 2: Determine the effect of partial reduction of glycogen synthesis once LD has developed.
Aim 3: Define if reactivation of the LD gene Epm2b (malin) in Epm2b-/- mice arrests LD progression.
Overlap: None

27. **What other organizations were involved as partners?**

i. **Organization Name:** University of Kentucky
ii. **Location of Organization:** Lexington, Kentucky, USA
iii. **Partner's contribution to the projec**t: Generated and provided fluorescent-androgens
    1. Financial support: None
    2. In-kind support: Same as 3
    3. Facilities: None
    4. Collaboration: Same as 3
    5. Personnel exchanges: None
    6. Other.

28. **SPECIAL REPORTING REQUIREMENTS**

a. DoD Award W81XWH-15-1-0409 is a post-doctoral training award and was not awarded to multiple investigators

b. **QUAD CHARTS:** Not applicable

29. **APPENDICES:** None